"Implementation of a high performance strategy for the detection and identification of small peptide hormones"

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PROJECT REVIEW

The use of small peptides as growth hormones releasing factors has become increasingly popular in the last few years. As described in the literature, a whole set of small peptides emerged these last decades representing today a more and more attractive class of performance-enhancing drugs for amateur and professional athletes. Several studies have reported the presence of these compounds in recent seized products confirming the illegal circulation of this class of substances.

The best analytical tool to detect these small peptides and also their analogs or discover unknown similar compounds is the high resolution / high accuracy mass spectrometry (LC-HRMS).

The aim of this study is to implement a high performance screening method by LC-HRMS in order to detect this new drug class. This project will focus on the available matrix from anti-doping tests: urine and blood (serum and plasma) but also on the seized products or internet-based drugs, which are the awareness keys of doping trends. Moreover, this project will also focus on the computer assisted method available with the LC-HRMS equipments. All the acquisition mode will be tested on the two technologies available in our laboratory (Q-Orbitrap and QqTOF) and an assessment of software helping to characterize metabolites without the reference material will be performed. The best strategy will be validated and applied to real samples from anti-doping controls.

Result and Conclusion:

A high performance screening procedure by LC-HRMS for prohibited peptides was developed and implemented in the laboratory. A protocol has been developed for urinary samples but also for plasma samples. Tests carried out show that the plasma method could also be easily applied to the serum samples. This screening protocol allows the detection of peptides < 2kDa but also peptides > 2kDa in a same method. The LOD obtained meet the WADA technical documents in force apart for larger peptides such as Tesamorelin, CJC-1295 or Sermorelin. For this category of peptides, another protocol should be applied in order to reach the expected LOD (0.5 ng/mL).

During this study, two kinds of instruments were evaluated: the QExactive+ system and the QTOF. The performances of the two instruments were globally equivalent but the QExactive+ instrument shows most of the time slightly better LODs and less background noise for some ion transitions. It was so retained as instrument of choice for drug testing routine analyses.

A stability study was performed during the validation process confirming that samples should be frozen as soon as possible after collection and stored frozen. A storage at +4°C is possible but should not exceed few days (3 days maximum for Alexamorelin or Goserelin for example).

Seized products and internet-based compounds were also analyzed and characterized thanks to comparison with a database build on the instrument. On the twelve internet/seized products at our disposal, three of them showed incorrect identification on the original vial. For example, one vial identified as HGH frag 176-191 contains exclusively Sermorelin and another vial identified as TB-500 was in fact Thymosin β4. The last case was a vial identified as TB-1000 (supposed to be Thymosin β4) but it contains a mixture of TB-500 and its metabolite TB-500 M3.